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Egg-Mass Gel of *Melanochlamys diomedea* (Bergh) Protects Embryos From Low Salinity

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Abstract. Many opisthobranch gastropods embed their embryos in gelatinous egg masses; however, the functions of gel are not well known. We analyze the hypothesis that egg-mass gel protects embryos from salinity change. Using egg masses of *Melanochlamys diomedea*, we found that experimental removal of gel decreased the ability of embryos to survive osmotic stress. We evaluate several possible protective mechanisms by estimating osmotic influx of water into egg masses and by modeling salt efflux from an egg mass. On immersion in low-salinity water, egg masses lost roughly 23% of their mass, indicating that osmotic influx of water did not occur. Therefore, the principal route of salinity change within the egg mass is probably salt efflux. The model suggests that this efflux occurs quite slowly even when ambient salinity changes rapidly. Slow salinity change may be less stressful for embryos because the mechanisms that regulate cellular volume have more time to adjust. We show experimentally that slow salinity changes are less harmful to veligers than rapid ones by isolating capsules from egg-mass gel and exposing them to gradual or abrupt salinity change. The results support the hypothesis that rate of change of salinity is an important determinant of embryo survival and that egg-mass gel retards the rate of salinity change.

Introduction

Many marine invertebrates embed their embryos in gelatinous masses. Because gel eliminates convective transport within masses, it slows the exchange of material between embryos and seawater. Reduced exchange may adversely affect embryos. For example, embryos in the centers of globose masses develop more slowly than those toward the edges, due to an inadequate supply of oxygen and the accumulation of waste (Booth, 1995; Chaffee and Strathmann, 1984; Strathmann and Strathmann, 1995). In other cases, however, reduced exchange may benefit embryos. For example, gel may retard the efflux of beneficial agents (salt water, heat) or the influx of damaging agents (fresh water, extreme heat, microorganisms). These kinds of benefits have been suggested, but little empirical work bears on them (Todd, 1981; reviewed by Pechenik, 1986). Gel may also protect embryos from predators and other environmental stresses, such as ultraviolet radiation, and may retain embryos in areas favorable for growth (Biermann *et al.*, 1992; Pechenik, 1986; Rumrill, 1990; Strathmann, 1985).

The egg masses of *Melanochlamys diomedea*, an opisthobranch gastropod, are particularly well suited to the study of the protective properties of gelatinous masses. Adult *M. diomedea* deposit masses in intertidal and shallow subtidal zones—on the bottoms of shallow, sandy lagoons and bays (Strathmann, 1987). These zones are exposed to a wide array of physical stresses. One common stress in the intertidal is low salinity, which may result from freshwater runoff (Drouin *et al.*, 1985; Sanders *et al.*, 1965) or rain (Gibbs, 1968; Tettelbach *et al.*, 1985). Low salinity can retard development or kill embryos, and even infrequent episodes of extremely low salinity may play an important role in the ecology and evolution of intertidal organisms.

This study is a four-part experimental and theoretical analysis of the hypothesis that egg-mass gel protects embryos from salinity change (Pechenik, 1982, 1983; Skoog, 1973; Thorson, 1946; Todd, 1981). (1) We tested the protective ability of the gel by taking advantage of egg-mass structure. Embryos are contained in capsules that are distributed throughout a gelatinous matrix; the cap-

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sules are easily removed from the gel by agitation. We exposed both embryos in gel and embryos removed from gel to a range of salinities and measured their survival. (2) We estimated the magnitude of osmotic influx of water into egg masses exposed to low-salinity water. (3) We evaluated several potential mechanisms of protection (Pechenik, 1983) by adapting a model of diffusion in a sphere (Crank, 1956) to salt efflux within and from egg masses. The model suggested several ways that gel may protect embryos from osmotic stress. One model prediction was that gel slows the rate of salinity change. (4) Using embryos isolated from gel, we experimentally tested the hypothesis that slow salinity change is less harmful than abrupt salinity change (Milne, 1938; Pechenik, 1982, 1983; Sanders *et al.*, 1965; Stickle and Ahokas, 1974). We discuss how our findings about salinity may apply to other substances that move into and out of egg masses by diffusion, and how transport considerations may bear on egg-mass design.

Materials and Methods

Egg masses

Opisthobranch egg masses consist of many clear capsules embedded within a gelatinous material, each capsule containing one to several embryos (Hurst, 1967). A typical *M. diomedea* egg mass is globose (Hurst, 1967), contains about 25,000 to 50,000 embryos (Lee and Strathmann, in press), and is tethered to the sediment by a strand of gel.

Egg masses were collected from False Bay, San Juan Island, Washington. The collection site is fed by a freshwater creek that may dramatically lower the salinity within the bay. During a low tide in April 1997 (after a period of rain), we measured a salinity of 0‰ near the middle of the bay. In the same area, we found four egg masses in which all embryos had died. Later in the *M. diomedea* breeding season (summer, fall), the creek flow rate is generally lower but may increase after rains.

Egg masses for experiments were collected during low tides in June 1995 and April and July 1997. We picked masses that had been laid recently, avoiding masses that were ragged, covered with diatoms, or in the late stages of hatching. Within 2 h of collection, the masses were placed in flow-through seawater tables (average temperature 11°C) at Friday Harbor Laboratories, San Juan Island. Several airstones were placed in the water near the masses to ensure adequate aeration. During experiments, all manipulated and unmanipulated egg masses were kept in small Pyrex or plastic bowls submerged in seawater in the tables.

Comparison of gelled and degelled egg capsules

All seawater used in the experiments was bag-filtered (10-μm pore size), and test salinities were diluted with fresh water purified by reverse osmosis. Salinities were measured with a hand-held refractometer (±1‰). Undiluted seawater (30‰) served as the control. The survival of embryos in unmanipulated egg masses and of embryos that had been removed from the gel was determined at five test salinities (0, 5, 10, 15, 30‰). We tested 18 egg masses, 9 degelled and 9 intact, at each of the five salinities, for a total of 90 masses.

To minimize age effects, egg masses for the experiment were limited to those containing relatively young embryos (pre-veligers without red "kidney spot," velum, or shell). To remove embryos from an egg mass (the "degelled" treatment), we cut the mass into small pieces, placed the pieces in a small glass vial containing 3 ml of seawater, shook the vial vigorously for 30 s, and filtered its contents through a 200-μm mesh (Nitex). The mesh retained large pieces of gel but allowed dislodged egg capsules to pass. For a subset of the manipulated egg masses, we checked egg capsules under a dissecting microscope (20×) to ensure that they were completely disaggregated and free of gel.

Immersion into the test salinity was abrupt. Capsules were poured onto a 64-μm mesh (Nitex), briefly rinsed with water at the test salinity, and washed into 50 ml of test water in a small Pyrex or plastic bowl. We patted dry the unmanipulated egg masses and placed them into 50 ml of the test water. Each bowl was agitated several times during the experiment to reduce boundary layers. After 3 h we transferred capsules and masses back to full-strength seawater by similar processes. Each egg mass, or the capsules from an egg mass, was counted as a replicate.

Two additional controls were necessary. First, the pH of fresh water (6.9; measured with a microelectrode) was lower than that of seawater (7.9). The seawater-dilution and freshwater treatments (15, 10, 5, 0‰) therefore experienced lower pH (7.9, 7.8, 7.6, 6.9, respectively). Low pH may adversely affect the development of some marine invertebrate larvae (Calabrese and Davis, 1966; Strathmann and Strathmann, 1995). To test the effects of pH, we brought some seawater to pH 6.9 with concentrated HCl. We degelled capsules of four additional masses, placed them in the acidic seawater for 3 h, then returned them to normal seawater. Second, the method used for separating egg capsules from the egg mass involves vigorous agitation, which may damage embryos so that they are more susceptible to osmotic stress. To test the effects of agitation, we degelled 15 masses by the method described above and 15 masses by a gentler method in which each mass was cut into small pieces and washed with a small amount of seawater. Dislodged capsules were picked out with a pipette. Capsules from each separation treatment were divided into three groups (capsules from five masses in each), and groups were subjected to 5, 10, or 30‰ by the method described above. After 3 h in the test salinity, capsules were transferred back to seawater.

Three days later we determined the survival rate for each egg mass. To standardize the scoring of manipulated and unmanipulated masses, we removed the egg capsules from the gel in the unmanipulated groups by the process described above. To score embryos as alive or dead, we examined 50–100 capsules from each mass under a compound microscope (100×). At this time almost all embryos in the control groups had become veligers (had developed red kidney spots, shells, velar lobes). We scored as alive those veligers having a red kidney spot and shell, showing no major deformities, and actively moving within the egg capsule. Dead individuals lacked several of the above characteristics and showed substantial tissue damage or loose debris in the egg capsule.

Movement of water between egg mass and environment

Fresh water may affect egg-mass size and shape by changing gel structure or by moving osmotically into the egg mass. We tested these possibilities by immersing 12 egg masses (collected in April 1997) in low-salinity water (5‰) for 3 h, and weighing them at 0, 10, 20, 30, 60, 90, 120, and 180 min. At each weighing, masses were blotted briefly and weighed to the nearest milligram. After 180 min, masses were returned to seawater and were weighed once more about 10 h later. Control masses were weighed on the same schedule but were kept in seawater for the duration of the experiment.

To examine more closely the mechanisms of weight change in egg masses, we performed a separate experiment to measure both fresh and dry weights of masses exposed to a variety of salinity conditions. Forty egg masses (collected in July 1997) were assigned haphazardly to four groups of ten. One group was exposed to low-salinity water (5‰) for 3 h, another to seawater for 3 h, another to low-salinity water for 3 h followed by seawater for 10 h, and another to seawater for 3 h followed by seawater again for 10 h. Masses were weighed (as described above) before and after the treatments, dried for 3 days at 60°C, and reweighed.

A model of salinity change at different locations in an egg mass

The non-steady-state diffusion of a substance from a sphere is described by the equation (Crank, 1956, p. 86)

$$c(r,\ t) = c_1 + (c_0 - c_1)\left(1 + \frac{2a}{\pi}\sum_{n=1}^{\infty}\frac{(-1)^n}{n}\sin\frac{n\pi r}{a}\,e^{-tDn^2\pi^2/a^2}\right)$$

where $c(r,t)$ is the concentration of diffusing substance at a distance r from the center of the egg mass at time t, c_1 is the initial uniform concentration of substance, c_0 is the surface concentration, a is the radius of the sphere, and D is the diffusion constant of the substance. We adapted this equation by making several assumptions: (1) Egg masses are spherical with $a = 0.566$ cm (see Results). This is not entirely accurate. Masses are on average 2.4 times longer than wide, which will result in model predictions that slightly underestimate the true rate of salinity change. (2) Seawater consists solely of sodium chloride and water. (3) The diffusion constant of sodium chloride, D, is the same in the gelatinous matrix as in water. We estimated D of sodium chloride in water (which diffuses as Na^+ and Cl^-) at 12°C as 1.043×10^{-5} cm^2/s (calculated from data in Lobo and Quaresma, 1989). The actual value of D may be lower, especially if the gelatinous matrix hinders movement of ions (or if ions adsorb onto the gel material). However, measured values of the diffusion constant of oxygen in *M. diomedea* egg masses are close to those of oxygen in water (Cohen and Strathmann, 1996). If D for sodium chloride is lower in the gel, the model will overestimate the rate of change of salinity. (4) Embryos and capsules do not affect diffusion through the mass. Gelatinous matrix makes up the largest fraction of the mass, approximately 93% by volume for freshly laid masses (calculated from unpublished data of C. E. Lee, University of Washington). This fraction probably changes somewhat as embryos develop, because both capsules and egg masses swell (Kress, 1971; H. A. Woods, pers. obs.). Even in late-stage egg masses, however, capsules are well-spaced (H. A. Woods, pers. obs.). If embryos did affect the diffusion constant, they would increase it (Hunter and Vogel, 1986)—leading us to underestimate the true rate of salinity change. (5) Masses experience no shape change or osmotic influx of water, only efflux of salt. If fresh water flows osmotically into masses (see Discussion), rates of salinity change will be underestimated. (6) Masses are in a well-stirred environment that reduces boundary layers to insignificance.

We wrote a computer program to calculate $c(r,t)$. The solution to $c(r,t)$ consists of an infinite series of terms, indexed by n. We approximated the solution by iterating each determination to $n = 1000$, which was sufficient for the calculation to converge to within a small fraction of a percent of the actual solution (checked in a subset of cases by iterating to $n = 10{,}000$). The profile of salinity change was calculated for a mass initially at 30‰ subjected abruptly to 5‰ ($c_0 = 5$). The effects of egg mass size (a) and distance from the center of the mass (r) were explored.

To determine the value of a (egg-mass radius), we measured 31 egg masses. Mass tethers were removed and the masses were laid on a petri dish. We measured lengths with a ruler (±1 mm) and circumferences at the widest point with a piece of string which we then measured with a ruler (Booth, 1995). After blotting each mass dry,

we weighed it (±0.01 g) and determined its volume (±0.05 ml) by the displacement of 5 ml of seawater in a 10-ml graduated cylinder (Booth, 1995).

Effects of gradual versus abrupt salinity change

We compared the survival of degelled embryos exposed to abrupt and gradual salinity changes. Ten egg masses were degelled, five with pre-kidney-spot embryos and five with kidney-spot veligers. The capsules from each of these masses were split into three groups: one group maintained in full-strength seawater for the duration of the experiment; a second group exposed abruptly to 5‰ for 1 h by the method described above; and a third group exposed to a gradual decline in salinity before exposure to 5‰ for 1 h. Egg capsules of the third group were initially in 5 ml of water in a 25-ml glass vial. Four times at intervals of 20 min we added 5 ml of fresh water to the vials. After each step, the measured salinity in the vial deviated from the calculated salinity by less than 1‰. Capsules were left at 5‰ for 1 h. During this time, after the capsules had settled to the bottom of the vial, we removed all except 5 ml of water. We increased the salinity to full strength (30‰) over 20 min in three steps by adding 1.65 ml and then 11.65 ml of full-strength seawater, then finally filtering the capsules into seawater. We scored individuals 6 days later, by which time all embryos had become veligers or died.

Results

Gelled versus degelled capsules

The effect of different salinities on gelled and degelled capsules is shown in Figure 1. The fraction of embryos surviving in all groups was uniformly high at 10‰ or greater. In the intact groups, the fraction surviving was also high at 5‰ (0.98) and only dropped off at 0‰ (0.31). In the degelled groups, survival was lower at 5‰ (0.19) and very low at 0‰ (0.04). At 5 or 10‰, cellular debris and internal parts were visible in the intracapsular fluid. In fresh water, the majority of tissues were severely degraded.

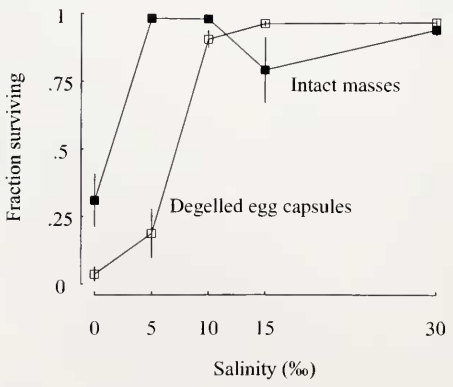

Figure 1. Survival of *Melanochlamys diomedea* embryos after immersion in various salinities for 3 h. Survival was scored 3 days after exposure to the test salinity. Filled squares (■) indicate embryos in egg masses, open squares (□) degelled embryos.

Table 1

Analysis of variance summary of the data, presented in Figure 1, for survival of Melanochlamys diomedea *embryos at various salinities*

Source	SS	df	MS	*F*	*P*
Salinity	15.99	4	4.00	57.3	<0.001
Egg-mass treatment (intact or degelled)	1.89	1	1.89	27.4	<0.001
Salinity*Mass trt.	4.87	4	1.22	17.64	<0.001
Residual	5.52	80	0.07		

These results indicate that gel is not necessary for protection at salinities above 10‰, assuming a 3-h exposure. The gel improved survival only when the salinity dropped below 10‰, but in this range the improvement was marked. The exceptionally large error bars on the gelled group at 15‰ were due to two probable outliers (survival of 0.24 and 0.06). It is possible that the embryos in these masses had died before they were collected. The data were transformed (angular) and differences were tested with a factorial analysis of variance. Both of the main terms (salinity and egg-mass treatment) were highly significant (Table 1). In addition, the interaction term was significant, indicating that low salinity killed the degelled group disproportionately (Fig. 1). The salinity that killed 50% of the embryos in each group—the LD50 (±95% CI)—was calculated with logistic regression (Venables and Ripley, 1994) after removal of the two outliers and the 30‰ groups. The LD50 of the degelled group (6.95 ± 0.20‰) was higher than that of the embryos in intact masses (1.17 ± 0.05‰).

Embryos exposed to low pH survived well (approx. 0.98), indicating that the lower pH of the more dilute groups in the main experiment did not confound the results. In addition, vigorous agitation did not make embryos more susceptible to osmotic stress. At the three test salinities (5, 10, and 30‰) of the control experiment, embryos in the agitated group survived at essentially the same rate (mean 0.04, 0.99, 1.0, respectively) as embryos in the "gentle" group (mean 0.02, 0.99, 0.99, respectively).

Water movement

Egg-mass weight declined rapidly during the first half hour of exposure to low-salinity water (5‰), then less

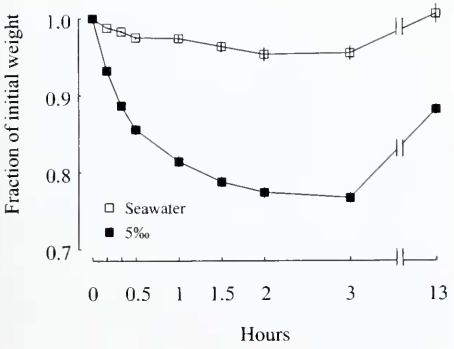

Figure 2. Fraction of *Melanochlamys diomedea* egg-mass weight remaining after various exposures to low-salinity water (5‰, ■) or seawater (□). After 3 h, the low-salinity masses were returned to seawater.

slowly before reaching a more-or-less constant level of about 77% of the initial weight (Fig. 2). During the subsequent 10 h in seawater, the masses regained much of their weight (88% initial weight). The control masses, in seawater for the duration of the experiment, also lost some weight (<5%) over 3 h, most likely by losing small bits of gel during blotting. The weights of the control egg masses also increased after 10 h, probably reflecting a gradual swelling during development (Kress, 1971; H. A. Woods, pers. obs.). In no case did an egg mass undergo any readily apparent change in shape.

A second experiment confirmed that egg masses exposed to low salinity lost wet weight, and that the weight was regained during subsequent exposure to seawater (Table II). In addition, low salinity affected dry weight. Egg masses exposed to low salinity for 3 h had a mean dry weight (0.005 ± 0.001 g) roughly a third of the mean dry weight of masses exposed to seawater for 3 h (0.015 ± 0.001 g). Masses exposed to low-salinity water for 3 h followed by seawater for 10 h, however, had a mean dry weight (0.017 ± 0.002 g), similar to the two other groups of masses exposed only to seawater (0.015 ± 0.001 and 0.019 ± 0.003 g).

Model and morphology

Table III shows the results of the morphological measurements of 31 egg masses. Together, average weight (0.75 g) and average volume (0.76 ml) indicate that the egg masses were nearly the same density as water. Although not spherical, the masses were only 2.4 times longer than wide. For the model, we estimated the radius, $a = 0.566$ cm, as the radius of a sphere having the same volume as the average measured volume (0.76 cm^3). We note that the masses (collected in early June) used for these measurements (Table III) were substantially larger than the masses collected (in late July) for the second water-movement experiment (Table II).

Figures 3 and 4 illustrate some model predictions. The salinities at three positions in an egg mass (near the middle, $r = 0.1$ cm; about halfway between center and edge, $r = 0.3$ cm; and near the edge, $r = 0.5$ cm) after 3 h of immersion in low salinity (5‰) were all predicted to be between 5 and 10‰ (Fig. 3). This range is one of high sensitivity for the embryos (Fig. 1); the model suggests that embryos near the middle ($r = 0.1$ cm) may have experienced slightly more tolerable salinities than embryos near the edge ($r = 0.5$ cm). The rate of change of salinity also depended on the position within the egg mass, with embryos near the edge experiencing higher rates. However, at all positions, the rate of change below 10‰ was quite slow.

The model also predicted that egg-mass size affects the rate of change of salinity (Fig. 4). Not surprisingly, the salinity in an egg mass 25% larger (by volume; $a = 0.609$ cm) changed more slowly than in one 25% smaller ($a = 0.514$ cm; salinity was modeled at $r = 0.3$ cm in both). However, the effect of a 25% increase or decrease in egg-mass volume was not as dramatic as the effect of position within the egg mass (Fig. 3).

Gradual versus abrupt salinity change

Both salinity treatment and embryo age significantly affected the fraction of embryos surviving (Fig. 5; 2-

Table II

Summary of egg-mass weights before and after exposure to low-salinity water (5‰), seawater, or both

Treatment	Initial fresh weight	Fresh weight after treatment	Dry weight after treatment	Dry weight as % of initial weight
3 h in seawater	0.37 ± 0.05	0.38 ± 0.05	0.015 ± 0.002	4.1 ± 0.1
3 h in 5‰	0.33 ± 0.05	0.28 ± 0.04	0.005 ± 0.001	1.4 ± 0.1
3 h in seawater + 10 h in seawater	0.36 ± 0.04	0.41 ± 0.05	0.017 ± 0.002	4.8 ± 0.1
3 h in 5‰ + 10 h in seawater	0.42 ± 0.07	0.45 ± 0.07	0.019 ± 0.003	4.7 ± 0.1

All weight are given in g ± SEM except last column (%). For each treatment, $n = 10$.

Table III

Morphology of Melanochlamys diomedea *egg masses* (n = *31*)

	Mean	SEM	Range
Length (cm)	2.25	0.32	1.5–3.8
Circumference (cm)	2.96	0.18	2.3–3.6
Volume (ml)	0.76	0.28	0.35–1.30
Mass (g)	0.75	0.29	0.34–1.32

factor ANOVA on angularly transformed data: $F = 37.9$, $P < 0.0001$ and $F = 40.6$, $P < 0.0001$, respectively). All embryos survived the control treatment well. Age affected the response to gradual and abrupt treatments. Veligers (dashed lines) survived better overall and also survived the gradual change (mean = 0.80) better than the abrupt change (0.30). The younger, pre-kidney-spot, embryos did not survive well under either the gradual (0.07) or abrupt (0.01) conditions of change. We note that, because individuals in different treatments were not completely independent (capsules within a mass were split into groups), ANOVA is not an entirely appropriate statistical test; however, no better test is available.

Discussion

Our observations demonstrate that gelatinous egg masses can protect embryos from salinity changes (Fig. 1). Although isolated embryos survived 3-h exposures to a wide range of salinities (10–30‰), gel markedly improved survival at the lowest salinities (0, 5‰). Gelatinous masses have not been previously examined for this protective ability, with the exception of the egg masses of a polychaete, *Scoloplos armiger* (Gibbs, 1968). The results parallel those presented here. Polychaete larvae within intact masses and those removed from egg masses survived sudden immersion in salinities as low as 14.4‰ (Gibbs, 1968). However, at an immersion salinity of 7.7‰, only polychaete larvae

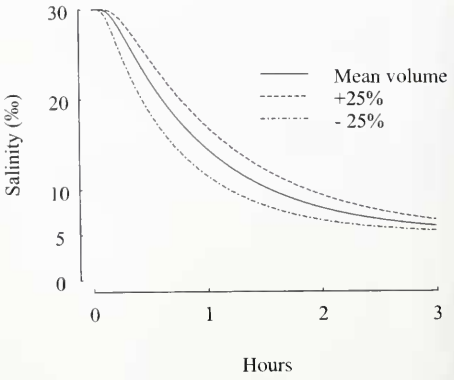

Figure 4. Model predictions of salinity change in egg masses of *Melanochlamys diomedea* as a function of time at a position about halfway between the center and the edge ($r = 0.3$ cm) of a spherical egg mass ($a = 0.566$ cm). The dashed lines indicate the trajectories at this same position ($r = 0.3$ cm) in a mass 25% larger ($a = 0.609$ cm) or smaller ($a = 0.514$ cm).

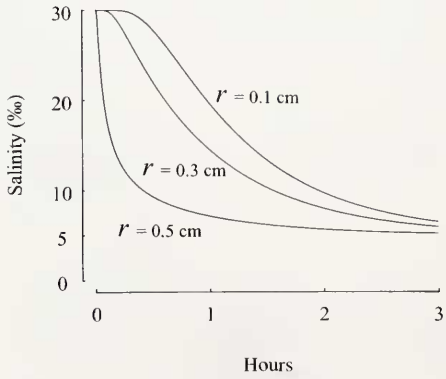

Figure 3. Model predictions of *Melanochlamys diomedea* egg-mass salinity as a function of time at three internal positions. The egg mass was assumed to be spherical with radius $a = 0.566$ cm. At the beginning of the simulation the salinity was 30‰ throughout the egg mass. The external salinity was held constant at 5‰.

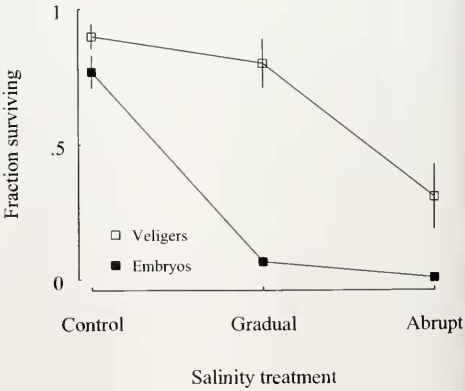

Figure 5. Survival of degelled embryos (■) and veligers (□) of *Melanochlamys diomedea* subjected to gradual or abrupt salinity change. Control refers to groups maintained in seawater for the duration of the experiment; gradual to embryos ramped to low salinity (5‰); and abrupt to embryos immersed immediately into low salinity (5‰).

inside the egg masses survived (0.67). No larvae survived immersion in fresh water.

Pechenik (1983) has suggested four possible mechanisms by which egg masses might protect embryos from salinity changes. Egg masses might (1) prevent the internal salinity from declining; (2) maintain an equilibrium concentration higher than ambient; (3) reduce the time spent at low salinity; (4) reduce the rate of change of salinity. The first and second hypotheses are unlikely in view of the mass structure. Gel consists predominantly of water in a matrix of mucopolysaccharides (Ghiselin, 1965; Todd, 1981), which would be unlikely to retain small solutes indefinitely. It is possible, however, that the matrix retains large, osmotically active molecules (Pechenik, 1983), or that the mucopolysaccharides themselves are osmotically active.

The third and fourth hypotheses may apply to *Melanochlamys diomedea*, but only if salinity within egg masses changes slowly. Our observations strongly suggest that this is the case. Egg masses do not experience a rapid osmotic influx of water. A rapid influx would cause egg masses to swell and gain weight; in contrast, we observed that the egg masses lost weight during the 3-h exposure to low salinity (Fig. 2). Replacement of high-density seawater by low-density, low-salinity water cannot explain these results, as the densities of seawater and low-salinity water differ by only about 2% (Horne, 1969). The weight loss suggests instead that, on contact with low-salinity water, the gel contracted and expelled some internal water—perhaps because the change in water activity altered the conformation of the gelatinous matrix (Parsegian *et al.,* 1995). An alternative explanation, breakdown and loss of the gel itself, is unlikely for several reasons. First, the difference in *dry weights* between egg masses exposed to seawater or low-salinity water (5‰) (Table II) can be accounted for largely by the difference in the salt content of seawater and low-salinity water. An egg mass weighing 0.38 g (fresh) would contain about 8 mg of salt if the internal water were seawater; a similar mass in low-salinity water (5‰)—assuming that it had shrunk to 77% its initial volume (Fig. 2)—would contain about 1 mg of salt. The difference between these values, 7 mg, is the larger part of the difference between the dry weights of masses exposed to seawater and low-salinity water for 3 h (10 mg, Table II). Second, comparison of the second and fourth treatments (Table II) shows that virtually all of the dry mass lost after a 3-h exposure to low salinity was regained during a subsequent exposure to seawater, an observation interpreted most simply as the influx of salt into an otherwise intact gelatinous matrix.

Because osmotic influx of water is not important, the principal mechanism of salinity change within the egg mass must be the efflux of salt. The model indicates that this efflux occurs quite slowly, especially as the concentration gradient becomes small (as the internal salinity falls below 10‰). The loss of salt will be accelerated somewhat by the decrease in the size of the egg mass (Fig. 2). In addition, smaller egg masses will experience higher rates of internal salinity change. However, as shown in Figure 4, even a 25% decrease in mass volume does not substantially change the trajectory of salinity change. Furthermore, we assumed that salt diffuses in gel as rapidly as it does in water. If salt diffuses more slowly in gel—a distinct possibility (see model assumptions above)—then the salinity would change even less rapidly than the model suggests.

Therefore, Pechenik's third hypothesis (above) may apply to *M. diomedea.* The maximum exposure to low salinity is set by the duration of the tidal cycle, about 6 h (exposure will be considerably shorter than this at sites close to the low tide mark). The model (Eq. 1, Figs. 3, 4) suggests that an egg mass requires more than 3 h to equilibrate with ambient salinities. In nature, therefore, the internal salinities of gel masses may decrease slowly enough that incoming tides rescue them before they equilibrate with low ambient salinity.

The fourth hypothesis (above) gains additional support from our experiments on veliger survival. We showed that veligers within egg masses survived a slow rate of salinity change better than an abrupt change (Fig. 5). We note that only veligers benefited from the gradual change (Fig. 5); embryos fared equally poorly in both gradual and abrupt changes. This may be due to the veligers' well-developed shells and opercular membranes (Thorson, 1946), which when closed, would retard the movement of solutes and water. Embryo survival may be improved by even slower salinity changes, especially between 10 and 5‰.

Why is the rate of change of salinity important to embryos? As salt leaves the egg mass, and the interior salinity falls, embryos will inhabit an increasingly hypoosmotic environment. Consequently, water will flow osmotically from the gelatinous matrix into the embryonic cells. The principal danger from this influx of water is cell swelling, which may inhibit cell function or, in extreme cases, damage or burst cells. Although intracellular solutes were not studied in *M. diomedea,* many marine invertebrates regulate the levels of these substances, especially free amino acids and some inorganic ions, in order to minimize osmotic concentration differences between cellular and external spaces (Amende and Pierce, 1980; Bedford, 1971; Chamberlin and Strange, 1989; Clark, 1985; Florkin and Schoffeniels, 1969; Greenwalt and Bishop, 1980; Kasschau, 1975; Pierce and Amende, 1981; Silva and Wright, 1994). But regulation of intracellular solutes is not instantaneous (Amende and Pierce, 1980; Burton, 1991; Neufeld and Wright, 1996; Silva and Wright, 1994). We speculate that regulation of cell volume operates over

the scale of minutes to hours in *M. diomedea,* and that egg mass gel protects by slowing the rate of salinity change so that cellular mechanisms of volume regulation are not overwhelmed.

Pechenik (1982, 1983) has examined another encapsulating structure, the egg capsule of prosobranch gastropods, for its ability to protect embryos from salinity change. Here the term "egg capsule" refers not to a structure similar to *M. diomedea* egg capsules (thin, permeable capsules embedded within the gel), but to a tough, leathery, semipermeable capsule that encloses both intracapsular fluid and embryos. Although capsule walls are thin (56 and 109 μm for two species of *Nucella*), they effectively protected embryos from osmotic changes comparable to those in this study (Pechenik, 1982). Considering the mechanisms listed above, Pechenik (1983) concluded that the second and fourth were likely to be important. The intracapsular osmotic concentration equilibrated to 25–50 mOsm/l above ambient osmotic concentration, and a gradual osmotic change improved the survival of embryos that had been removed from their capsules. Thus, while prosobranch capsules and opisthobranch masses both protect embryos from osmotic change, the mechanisms may be different: prosobranch capsules resist salinity change with semipermeable membranes, whereas opisthobranch masses resist by retaining relatively large volumes of water and exploiting the slowness of diffusion.

A challenging problem in the study of taxa that lay gelatinous egg masses is to explain the interspecific diversity of egg-mass sizes and shapes, which range from thin sheets or coils to small, tethered spheroids to large, sausage-shaped masses (Booth, 1995; Hurst, 1967; Strathmann, 1987). Our findings, and those of others, suggest that gel has a profound effect on exchange processes between the interior of the egg mass and the environment. For example, embryonic oxygen requirements may impose an upper limit on the size of egg masses (Strathmann and Strathmann, 1995; but see Booth, 1995). An egg mass optimized for oxygen delivery should therefore be relatively small. On the other hand, the present study suggests that smaller masses are less able to protect from salinity change. The size and shape of egg masses may be determined in part by compromises between maximizing rate of oxygen delivery and maximizing protection from salinity change. The importance of various exchange processes will depend on the environment (for example, subtidal species may not be affected by salinity changes). Of course, other gel functions not related to exchange, such as protection from predators, retention of embryos in favorable sites, and protection from ultraviolet radiation, may also be important (Biermann *et al.,* 1992; Pechenik, 1986; Rumrill, 1990; Strathmann, 1985). Further physiological, ecological, and comparative studies will be necessary to determine the actual importance of various gel functions.

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